# SECONDARY REFERENCE MATERIALS FOR METHOD CONTROL: A WAY OF APPRECIATING THE ACCURACY OF RESULTS

**The standard ISO 5725-6 : 1994 (Paragraph 4.2.3: Comparison with a reference value for a laboratory) offers a way of appreciating the accuracy of results during the analysis of reference materials: the calculation of the critical difference with a 95% probability level. This calculation is based on the technical performances of the analytical method described in the corresponding normative documents (reliability parameters: repeatability and reproducibility).**

It can be calculated as follows:

That is to say, $\bar{y}$, the mean of n test results (under repeatability conditions) on a SRM presenting a reference value of $\mu_0$. The critical difference, CD, for $(y-\mu_0)$ is determined according to the standard ISO 5725-6: 1994 (paragraph 4.2.3) as follows:

$$CD = (1/\sqrt{2})\sqrt{[(2.8\,\sigma R)^2 - (2.8\,\sigma r)^2\,((n-1)/n)]}$$

σr: standard deviation of repeatability
σR: standard deviation of reproducibility
n: number of determinations of the SRM

## 1) Example in microbiology: TOTAL GERMS SRM

**Reference document ISO 4833: 2003 –** Food Microbiology – Horizontal method for enumeration of micro-organisms – Colony counting technique at 30°C-

In milk: Repeatability: **r = 0,25 log**
Reproducibility: **R = 0,45 log**

• **Case of duplicate analyses of SRM:**

**CD = 0.293 log**

*In % CFU/ml, in comparison to the reference:*
*+96% for superior values*
*-49% for inferior values*

For a SRM reference value of 100 000 germs/ml or 5.00 log

The mean log values of the two analyses must be between 4.71 log (-49%) et 5.29 log (+96%).

If, for example, the values obtained are respectively 45 000 and 50 000, two calculations are possible:
1) Log values obtained are 4.65 and 4.70 of which the mean is 4.68 < 4.71 therefore not acceptable.
2) *For the original values in CFU ml: the inferior limit is –49% of the reference value, that is 51 000. The mean of the two results is 48 000, which is inferior to 51 000 therefore not acceptable.*

• **Case of a single SRM analysis:**

**CD = 0.318 log**

*In % CFU/ml, in comparison to the reference:*
*+108% for superior values*
*-52% for inferior values*

For a SRM reference value of 100 000 germs/ml or 5.00 log

The log value obtained must be between 4.68 log and 5.32 log.

If, for example, the values obtained are respectively 45 000 and 50 000, two calculations are possible:
1) 50 000 (4.70 log) is acceptable; 45 000 (4.65 log) is not acceptable.
2) *For the original values in CFU/ml: the value obtained must be between 48 000 (-52%) and 208 000 (+108%). 50 000 is acceptable; 45 000 is not acceptable.*

## 3) Example in chemistry: MILK DRY MATTER SRM

**Reference document NF V 04 367 : 1985 –** Milk, cream and unsweetened concentrated milk: Determination of dry matter

In milk: Repeatability: **r = 0.10g / 100g**
Reproducibility: **R = 0.20g / 100g**

• **Case of duplicate analyses of SRM:**

**CD = 0.13g / 100g**

For a SRM reference value of 13.00g / 100g, the mean of two analyses must be between 12.87 et 13.13g / 100g

• **Case of a single SRM analysis:**

**CD = 0.14g / 100g**

For a SRM reference value of 13.00 g /100g, the observed value must be between 12.86 et 13.14 g/100 g

This approach is an existing possibility for the exploration of reference material test results, based on the reliability characteristics of the tested method, described in the corresponding normative documents. It allows a tolerance level to be fixed, beyond which a search for the causes can be engaged and corrective action can be set up. The prerequisite for this approach is a good adequacy of the reliability values with the veritable performance of the method. In all new publications since about 2000, the values are calculated according to the ISO protocol corresponding to CECALAIT's normative sector (8 laboratories from at least two countries, 6 representative samples from the domain of application in blind duplicate and calculated according to the standard ISO 5725 parts 1 et 2). For the older documents, it is better to stay vigilant on the pertinence of the reliability values, and therefore the critical differences obtained by help of this calculation method. It could be thought that this standardised approach is succeeded by the calculation of a maximum limit value, which it would not seem opportune to exceed in this type of application. However, according to the case: calibration and verification of an instrumental method for example, other methods of calculation aiming at the determination of a tolerance level could be set up by the laboratories, as much as it corresponds to their needs.